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5-Fluorouracil–cisplatin adducts with potential antitumor activity

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Abstract

Using 5-fluorouracil (5-FU) and *cis*-diamminedichloroplatinum(II) (cisplatin, CDDP) as starting compounds, 5-FU–cisplatin adducts *cis*-[Pt(NH_3)$_2$(HFU)Cl] (**1**) and *cis*-[Pt(NH_3)$_2$(HFU)$_2$] (**2**) were prepared. The obtained complexes were characterized by IR, ES-MS and ^{1}H NMR spectroscopy. Complex **1** reacted with guanosine-5′-monophosphate (5′-GMP) and gave rise to a stable mixed-ligand complex *cis*-[Pt(NH_3)$_2$(HFU)(GMP)] (**3**), whereas **2** did not undergo a similar reaction. In vitro cell growth inhibition tests of complexes **1** and **2** exhibited moderate antitumor activities against the melanoma B16-BL6 cell line. This work provides the basis for a potential alternative for the combinational use of 5-FU and CDDP in cancer therapy.
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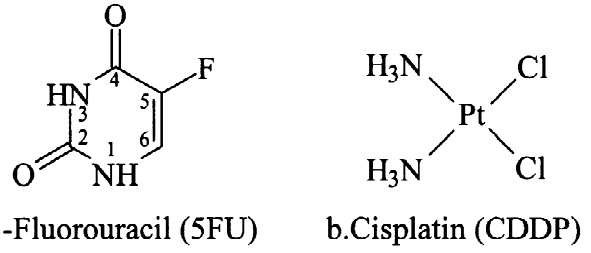
Keywords: 5-Fluorouracil; Cisplatin; Complex; Antitumor activity

1. Introduction

Anticancer drugs are rarely used singly to treat cancer, because only a few tumors are sensitive enough to be cured by single drugs. For a specific type of tumor, effective chemotherapy usually depends on suitable combinations [1]. Many combinations in clinical use consist of an antimetabolite with one or more other anticancer agents.

Antimetabolite 5-fluorouracil (5-FU) (Fig. 1a) is one of the major anticancer agents used clinically for the treatment of stomach, colorectal, head and neck cancers. However, response rate and duration have limited its efficacy, although some 5-FU dosing schedules such as continuous infusion have increased the response rate compared to bolus injection [2]. Many efforts have been made to potentiate the antitumor activity of 5-FU, and chemical and biochemical modulations of 5-FU by combination with cytotoxic or noncytotoxic agents are among the main strategies [3].



a. 5-Fluorouracil (5FU) b.Cisplatin (CDDP)

Fig. 1. Structural drawing of 5-FU (a) and CDDP (b).

Cisplatin (CDDP) (Fig. 1b) is one of the most widely utilized antitumor drugs for the treatment of testicular, ovarian and many other cancers [4]. However, due to the severe toxicity such as nephrotoxicity, neurotoxicity, and emetogensis, the application of CDDP has been limited to a certain extent [5]. Moreover, CDDP has to be administered in a relatively high dose, because more than 80% of injected drug binds to serum albumin, and albumin-bound CDDP is thought to be inactive [6]. These drawbacks have stimulated researches that could potentiate the effectiveness of CDDP.

Both 5-FU and CDDP are DNA-interacting agents, and combination of these two agents may lead to decreased repair of DNA damage or increased DNA adduct formation. In fact, many preclinical and clinical studies have indicated a potential beneficial effect of the combination of 5-FU and CDDP [7]. For example, continuous infusion of 5-FU with CDDP is more effective than each drug alone in head and neck cancer [8–14] and colorectal cancer [15,16]. Although the combination of 5-FU and CDDP varies, it

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remains a physical procedure rather than a chemical one. So far, no attempt has been made to chemically join these two anticancer drugs to form an integrated entity. Decades ago, Lippert et al. thoroughly investigated the platinum complexes of uracil and 1-methyluracil [17,18], however, the antitumor activity of those platinum pyrimidine-2, 4-dione-complexes remains unknown. In this work, we have synthesized two complexes where 5-FU is covalently attached to the $\{Pt(NH_3)_2Cl\}^+$ or $\{Pt(NH_3)_2\}^{2+}$ moieties. The potential DNA-binding properties of the complexes were investigated using the model compound 5′-GMP. The in vitro antitumor activity against melanoma B16-BL6 cells was also reported in this work.

2. Experimental

2.1. Materials and methods

Solvents such as dimethylformamide (DMF), diethylether and common reagents such as KOH, $AgNO_3$ are all of analytical grade and used as received. $K_2[PtCl_6]$ was obtained from Shanghai First Reagent Factory, 5-FU from Acros, and the disodium salt of 5′-GMP from Sigma. $K_2[PtCl_4]$ was synthesized according to the reported procedure via reduction of $K_2[PtCl_6]$ by hydrazine dihydrochloride [19]. *cis*-$[Pt(NH_3)_2Cl_2]$ (CDDP) and *cis*-$[Pt(^{15}NH_3)_2Cl_2]$ were prepared from $K_2[PtCl_4]$ according to a method described previously [20,21].

Infrared spectra were recorded on a Bruker VECTOR22 spectrometer as KBr pellets (4000–450 cm^{-1}). Elemental analyses were performed on a Perkin-Elmer 240C analytical instrument. Electrospray mass spectra were recorded using an LCQ electron spray mass spectrometer (ES-MS, Finnigan) by loading 1.0 μl of solution into the injection valve of the LCQ unit and then injecting into the mobile phase solution (50% of aqueous methanol) that was carried through the electrospray interface into the mass analyzer at a rate of 200 μl min^{-1}. The voltage employed at the electrospray needles was 5 kV, and the capillary was heated to 200 °C. A maximum ion injection time of 200 ms along with 10 scans was set. Positive or negative ion mass spectra were obtained. The predicted isotope distribution patterns for each of the complexes were calculated using the ISOPRO 3.0 program [22]. The ^1H NMR spectra were recorded at 298 K on a Bruker DRX-500 spectrometer using standard pulse sequences. The two-dimensional [^1H, ^{15}N] heteronuclear single-quantum coherence (2D [^1H, ^{15}N] HSQC) NMR spectra were recorded as previously described [23].

2.2. Preparations

2.2.1. K(HFU)

K(HFU) was prepared by a procedure analogous to that reported for the thymine salt [24]. Speciafically, an equimolar (5 mmol) amount of 5-FU and KOH was added to 10 ml water and the suspension was stirred for 3 h at room temperature. After filtration, the filtrate was evaporated to dryness to obtain a white powder. Then, excess of DMF (20 ml) was added to it and stirred for 30 min at 90 °C before cooling to room temperature. The salt was filtered off from the DMF solution and dried in vacuo. Yield: 89%. Anal. Calc. for $C_4H_2N_2O_2FK$ (168.17): C: 28.56; H: 1.19; N: 16.65%. Found: C: 28.58; H: 1.18; N: 16.60%. IR (KBr pellet): see Table 1 for details.

2.2.2. cis-[Pt(NH$_3$)$_2$(HFU)Cl] (**1**)

This complex was prepared by the following procedure: first, an equimolar equivalent (0.5 mmol) of CDDP and $AgNO_3$ in 10 ml DMF were stirred for 40 h at ambient temperature. The formed AgCl was filtered off, and a yellowish solution containing *cis*-$[Pt(NH_3)_2(DMF)Cl]NO_3$ was obtained. The reaction was performed in the dark. To this solution, one molar equivalent of K(HFU) (0.5 mmol) was added and the suspension stirred for 3 days at ambient temperature. KNO_3 was filtered off and an excess amount of diethylether was added to the yellowish filtrate and a white precipitate was formed immediately. The solvent was removed by centrifugation and the white precipitate obtained was dried in vacuo. Yield: 80%. Anal. Calc. for $C_4H_8N_4O_2FClPt$ (393.67): C: 12.20; H: 2.05; N: 14.23%. Found: C: 12.98; H: 2.20; N: 15.19%. ^1H NMR (500 MHz, D_2O, 298 K): δ 7.67d, 7.76d. IR (KBr pellet): see Table 1 for details.

cis-$[Pt(^{15}NH_3)_2(HFU)Cl]$ was prepared by the same procedure starting from *cis*-$[Pt(^{15}NH_3)_2Cl_2]$, however, *cis*-$[Pt(^{15}NH_3)_2(HFU)_2]$ was also formed as shown in the 2D [^1H, ^{15}N] HSQC NMR spectra (see 3.3 for details).

Table 1
IR frequencies and assignments of 5-FU, CDDP and their adducts (KBr pellet, cm^{-1})

	$\nu_a(NH_3)$	$\nu_s(NH_3)$	ν (CO)	δ_a(HNH)	δ_s(HNH)	$\rho_r(NH_3)$	ν (PtN)
5-FU	ν(NH): 3420–3100		1670, 1720	δ(NH): 1430, 1300	ρ_r(NH): 810		/
K(HFU)	ν(NH): 3426		1655, 1592	δ(NH): 1409, 1279	ρ_r(NH): 818		/
CDDP	3280	3195	/	1549, 1620	1300	790	510
1	3446	3299	1666, 1610	1478	1384	840	493
2	3446	3279	1764, 1659	1521	1384	826	488

Abbreviation: HFU, 5-fluorouracil monoanion.

*2.2.3. cis-[Pt(NH$_3$)$_2$(HFU)$_2$] (**2**)*

This was synthesized in a way similar to **1**, except that the molar ratio between CDDP and $AgNO_3$ and K(HFU) was changed to 1:2:2. Yield: 60%. Anal. Calc. for $C_8H_{10}N_6O_4F_2Pt$ (487.28): C: 19.71; H: 2.06; N: 17.25%. Found: C: 20.42; H: 2.30; N: 18.02%. ^{1}H NMR (500 MHz, D_2O, 298 K): δ 7.38d, 7.50d, 7.51d, 7.57d, 7.60d, 7.61d, 7.68d. IR (KBr pellet): see Table 1 for details.

2.3. Reaction of complexes *1* and *2* with 5′-GMP

One molar equivalent amount of **1** (or **2**) and 5′-GMP (0.005 mmol) were added to 0.5 ml D_2O, the mixture was incubated at 37 °C in the dark for 24 h before the ^{1}H NMR spectra being recorded.

The reaction of *cis*-[Pt($^{15}NH_3$)$_2$(HFU)Cl] with 5′-GMP was carried out in 90% water+10% D_2O (0.5 ml) in a NMR tube at 310 K: *cis*-[Pt($^{15}NH_3$)$_2$(HFU)Cl] (0.005 mmol)+5′-GMP (0.005 mmol). The 2D [^{1}H, ^{15}N] HSQC NMR spectra were recorded immediately after mixing the two reactants and the reaction was followed for 30 h.

2.4. Cytotoxic activity

Melanoma B16-BL6 tumor cells were grown in RPMI 1640 medium supplemented with 10% freshly inactivated fetal calf serum (FCS) and antibiotics. The solutions of complexes **1** and **2** were freshly prepared with the RPMI 1640 medium, and diluted to the required concentration with culture medium when used. The cells harvested from exponential phase were seeded equivalently into a 96-well plate (2×10^5 per ml, 100 μl per well); then the compounds studied were added in a concentration gradient to give final concentrations at 1×10^{-4}, 1×10^{-5}, 1×10^{-6} and 1×10^{-7} M, respectively. The plates were kept at 37 °C in a humidified atmosphere of 5% CO_2 and incubated for 24 and 48 h respectively; then MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] solution of an appropriate concentration (2 mg/ml) was added to each well (40 μl) and the plates incubated at 37 °C for 4 h. The measurements of absorbance of the solutions related to the number of live cells were performed on an ELISA spectrophotometer at 540 nm [25]. The following formula was used to evaluate the drug efficacy against the tumor cells: inhibition rate (%)=[($OD_{control}-OD_{drug}$)/$OD_{control}$]×100. The IC_{50} values were derived from semilog plots of percentage control versus drug concentration using the Logit method and defined as the drug concentration that resulted in a 50% reduction in cell numbers compared to untreated controls.

3. Results

3.1. IR spectra

The characteristic vibrations of NH_3 and C=O groups for complexes **1** and **2** are listed in Table 1. In order to compare IR spectral changes between original drugs and the complexes, the absorption frequencies of 5-FU and CDDP are also listed.

The strong characteristic absorptions of C=O groups of 5-FU and K(HFU) were observed between 1600 and 1720 cm^{-1}, while the NH_3 groups of CDDP gave a broad absorption band in the 3000–3600 cm^{-1} region. After the reaction of 5-FU with CDDP, the absorptions for both NH_3 groups and C=O groups shifted to higher wave numbers (3279–3446 cm^{-1} for NH_3 and 1610–1764 cm^{-1} for C=O), which suggested the formation of adducts. Absorptions at 493 cm^{-1} for complex **1** and 488 cm^{-1} for complex **2** can be assigned to the Pt–N bonds.

3.2. ES-MS spectra

ES-MS spectra of **1** and **2** were recorded in aqueous solution and the data are summarized in Table 2. The calculated molecular masses and the isotopic distribution of these peaks matched perfectly with the corresponding formulas.

When complex **1** first dissolved in water, only one peak at 430.9 was observed, which can be assigned to [Pt(NH_3)$_2$(HFU)Cl]+K^+. After 30 min, two new peaks appeared at 358.1 and 375.0 (Fig. 2). The former can be assigned to one positively charged [Pt(NH_3)$_2$(HFU)]$^+$ (**1′**), which was unstable and converted to [Pt(NH_3)$_2$(HFU)(H_2O)]$^+$ (**1″**) (375.0) afterwards. The hydrolytic pathway of complex **1** is shown in Scheme 1. Complex **2** gave a single peak at 526.1 arising from [Pt(NH_3)$_2$(HFU)$_2$]+K^+, which is stable in solution and does not change with time.

Fig. 3 shows ES-MS spectrum for the reaction product

Table 2
Observed and calculated molecular masses for the complexes **1**, **2**, **3**

Complex	Formula	Constitution	Observed mass[a]	Calculated mass
1	[Pt(NH_3)$_2$(HFU)Cl]+K^+	$C_4H_8N_4O_2FClKPt$	430.9–436.8	432.8
1′	[Pt(NH_3)$_2$(HFU)]$^+$	$C_4H_8N_4O_2FPt$	357.2–361.3	358.2
1″	[Pt(NH_3)$_2$(HFU)(H_2O)]$^+$	$C_4H_{10}N_4O_3FPt$	375.0–379.0	376.2
2	[Pt(NH_3)$_2$(HFU)$_2$]+K^+	$C_8H_{10}N_6O_4F_2KPt$	525.0–529.1	526.4
3	[Pt(NH_3)$_2$(HFU)(GMP)Na]$^+$	$C_{14}H_{21}N_9O_{10}FNaPPt$	742.1–746.9	743.4

[a] The peaks are separated by 1 m/z in the mass region indicated.

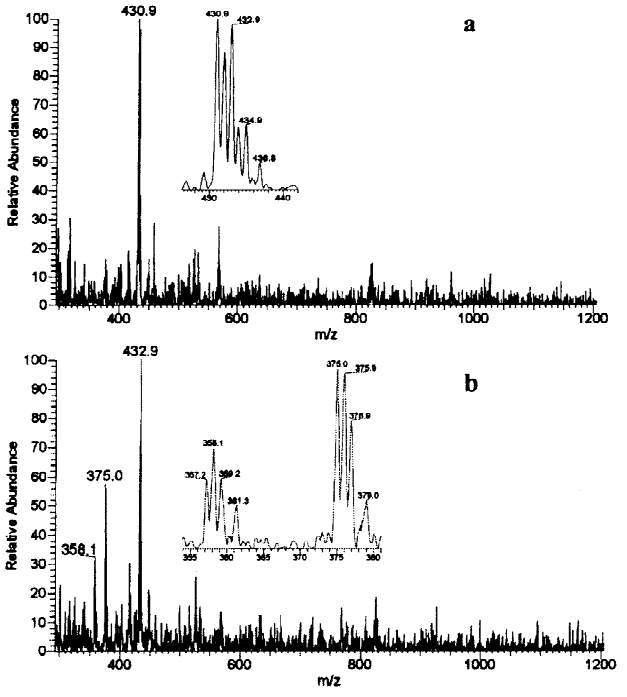
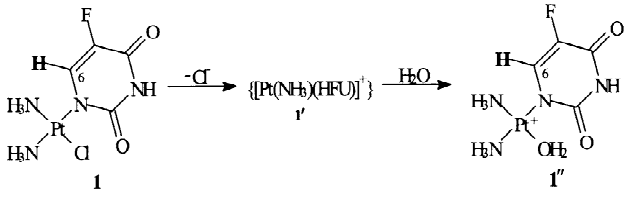


Fig. 2. ES-MS spectra of complex **1** (a) and its hydrolyzed species (b) in aqueous solution.



Scheme 1. Hydrolytic pathway of complex **1** in aqueous solution.

of complex **1** and 5′-GMP. The major peak detected at 743.0 can be assigned to the positively charged compound $\{[Pt(NH_3)_2(HFU)(GMP)]+Na^+\}$ (**3**). A peak at 1462.9 was also observed which indicates the presence of the dimer of complex **3** $\{[Pt(NH_3)_2(HFU)(GMP)]_2+Na^+\}$. The dimer form is often observed during ES-MS experiments—it may be formed in the capillary due to the high temperature and pressure. The structure of the dimer is unknown. It may be a pair of parent molecules connected via H-bonding, van der Waals force or electrostatic interactions.

3.3. NMR spectra

The major ^{1}H NMR peaks of complexes **1**, **2** and **3** in D_2O and their assignments are listed in Table 3. Chemical shift changes of H6 on 5-FU before and after reactions are shown in Fig. 4. Apart from the major peaks, the ^{1}H NMR spectra of **1**–**3** also showed the presence of minor amounts of DMF [$\delta(CH_3)$ 2.78, 2.93; δ(HCO) 7.85].

The H6 signal of free 5-FU appears as a doublet at 7.58 ppm in ^{1}H NMR spectrum, due to its coupling with ^{19}F ($^3J_{^1H,^{19}F}$=6 Hz) (Fig. 4a). It shifted once coordinated to Pt(II) as shown in Fig. 4b and c. For complex **1**, initially only one doublet at 7.76 ppm was observed, while a new peak at 7.66 ppm appeared after 30 min. In solution, the new signal could be attributed to the hydrolyzed species of complex **1** where one Cl^- is replaced by H_2O. For complex **2**, however, the spectrum is much more complicated—nine signals were observed in the H6 region (Fig. 4c).

The 2D [^{1}H, ^{15}N] HSQC NMR spectra were recorded to monitor the reaction of ^{15}N-labelled complex **1** with 5′-GMP during 24 h. Selected spectra showing the changes with time and the corresponding assignments for major cross-peaks are shown in Fig. 5 (Table 4).

3.4. Cytotoxic activity

The cytotoxic activities of complexes **1** and **2**, together with 5-FU and CDDP against melanoma B16-BL6 cells have been tested. The results are summarized in Fig. 6. and Table 5, respectively. Both complexes **1** and **2** are sig-

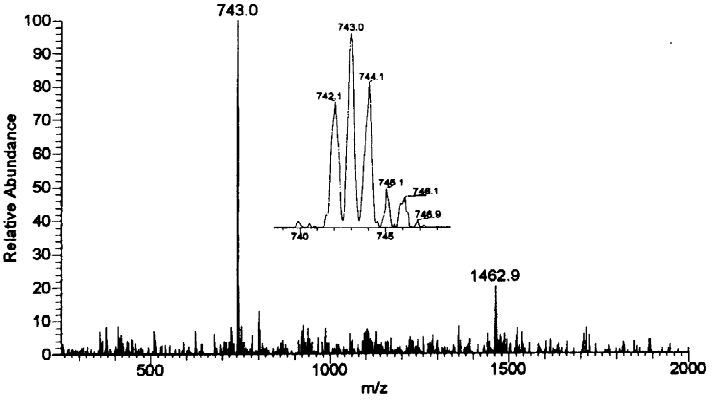


Fig. 3. ES-MS spectrum of 5′-GMP adduct of complex **1**.

Table 3
^{1}H NMR chemical shifts of 5-FU and complex **1**, **2, 3** (500 MHz, D_2O, 298 K)

Complex	Chemical shift (ppm)	Assignments
5-FU	7.58d	H6 (5-FU)
1	7.67d, 7.76d	H6 (5-FU)
2	7.38d, 7.50d, 7.51d, 7.57d, 7.60d, 7.61d, 7.68d	H6 (5-FU)
3	7.59d, 7.63d, 7.72d	H6 (5-FU)
	3.91m; 4.18d; 4.25m; 5.81d, 8.60s	H2′, H3′; H5′; H4′; H1′; H8 (GMP)

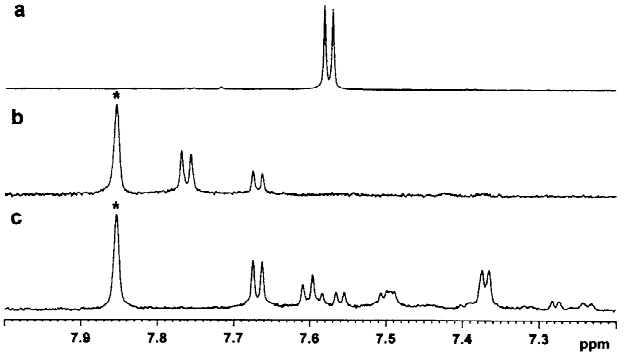


Fig. 4. ^{1}H NMR signals of H6 (5-FU): (a) before reaction; (b) in complex **1**; (c) in complex **2**. The peak with the asterisk arises from **H**CO of DMF.

nificantly less active compared with 5-FU and CDDP. However, complex **2** possesses a considerable inhibition effect (>40%, 48 h) against melanoma B16-BL6 cells even at a concentration of 10^{-7} M (Fig. 6). However, the activities of adducts are somewhat lower than that of 5-FU and CDDP at most of the concentrations. At all concentration ranges, the antitumor activities of adducts are more similar to that of 5-FU than to CDDP.

4. Discussion

Though the ^{1}H NMR spectrum showed up a complicated character (Fig. 4c), the composition of complex **2** shown by the ES-MS data is certain. It has been reported that the coordination sites of the unsubstituted uracil could vary with the solvent, the pH, the temperature, and the Pt:uracil ratio [17]. This case may fit for 5-FU too. Therefore, the different signals in ^{1}H NMR are likely due to the formation of HFU–N1 and HFU–N3 tautomer complexes. These tautomers could also give rise to peaks appeared in ^{1}H NMR (Fig. 4c) and 2D [^{1}H, ^{15}N] HSQC NMR spectra (Fig. 5).

The DNA binding property of complexes **1** and **2** was probed by their reactions with the model compound 5′-GMP (Fig. 7). As shown by ^{1}H NMR, the H8 signal of

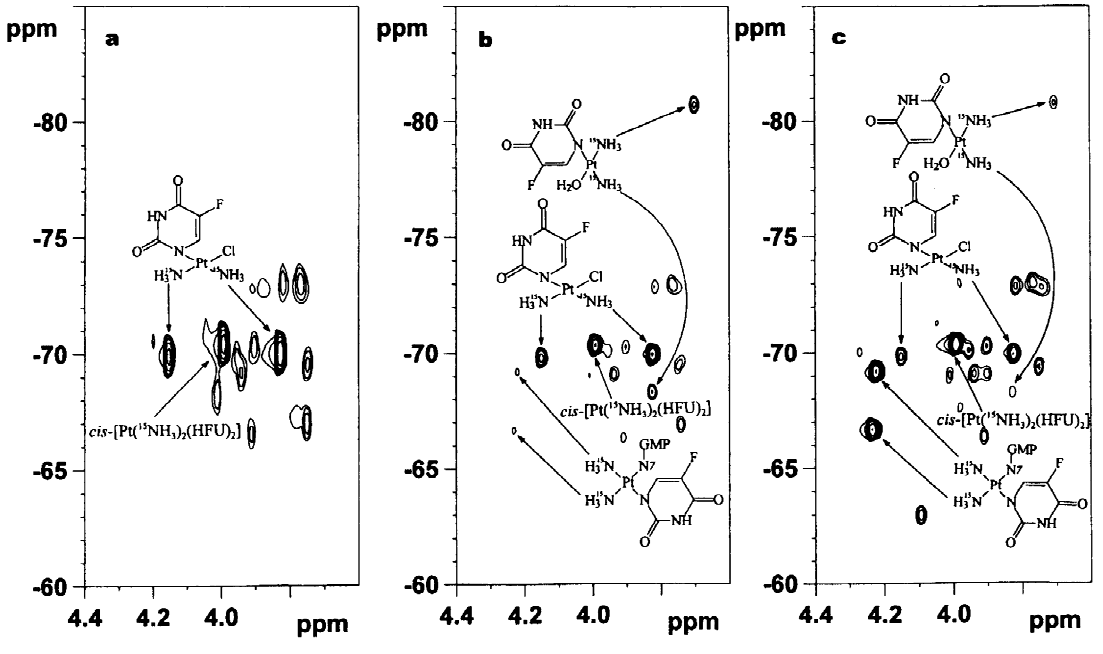


Fig. 5. 2D [^{1}H, ^{15}N] HSQC NMR spectra recorded before the reaction of ^{15}N-labelled complex **1** with 5′-GMP (a) and after it had progressed 45 min (b) and 21 h (c).

Table 4
^{1}H and ^{15}N NMR chemical shifts for ^{15}N-labelled complex **1**, **1′**, **2** and **3** (500 MHz, 90% H_2O + 10% D_2O, 310 K)

	δ (^{1}H) (ppm)	δ (^{15}N) (ppm)
cis-[Pt($^{15}NH_3$)$_2$(HFU)Cl]	3.825	−69.936 (*trans* N)
	4.154	−69.888 (*trans* Cl)
cis-[Pt($^{15}NH_3$)$_2$(HFU)$_2$]	3.992	−70.326
cis-[Pt($^{15}NH_3$)$_2$(HFU)(H_2O)]$^+$	3.702	−80.678 (*trans* O)
	3.824	−68.258 (*trans* N)
Cis-[Pt($^{15}NH_3$)$_2$(HFU)(5′-GMP-*N7*)]$^+$	4.220	−69.176 (*trans* N, 5-FU)
	4.230	−66.658 (*trans* N, GMP)

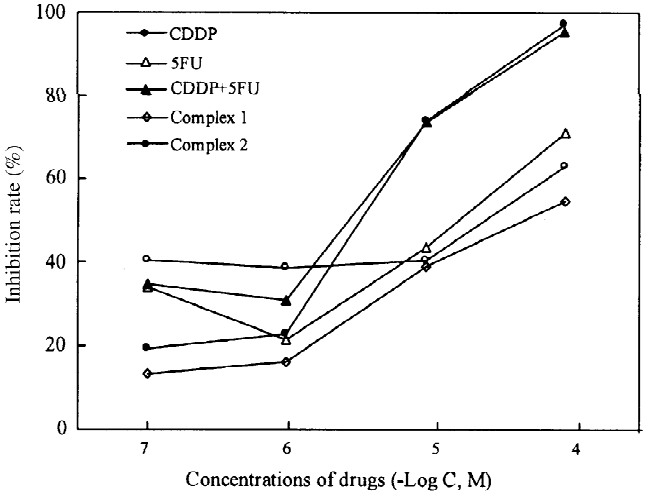
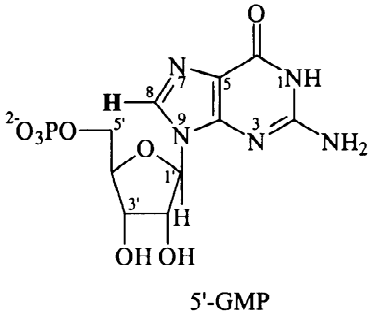


Fig. 6. Inhibition rate of complexes **1** and **2** for proliferation of melanoma B16-BL6 cells (with 5-FU and CDDP for comparison). B16-BL6 cells (2×10^4/well) were incubated with drugs as shown concentrations for 48 h. The proliferation of B16-BL6 cells was determined by the MTT method.

Table 5
In vitro antitumor activity against melanoma B16–BL6 cells (IC_{50})

Compound	IC_{50} (M)
5-FU	1.43×10^{-6}
CDDP	4.07×10^{-6}
CDDP + 5-FU	2.56×10^{-6}
Complex **1**	$>10^{-4}$
Complex **2**	9.98×10^{-5}



5'-GMP

Fig. 7. Guanosine-5′-monophosphate (5′-GMP).

5′-GMP shifted from 8.16 ppm to 8.60 ppm upon binding with complex **1** which indicated N7 coordination [26–30]. However, under similar reaction conditions, complex **2** does not react with 5′-GMP because no shift of ^{1}H signal of 5′-GMP was observed. Complex **2** does not bind to 5′-GMP as demonstrated by NMR and ES-MS, therefore, 5-FU cannot be replaced by 5′-GMP. However, complex **1** binds readily to 5′-GMP and forms a model monofunctional adduct.

Due to the complexity of the ^{1}H NMR, especially for complex **2**, 2D [^{1}H, ^{15}N] HSQC NMR spectroscopy was applied for the study as it has been proved to be a powerful method to monitor the reactions of ^{15}N-labelled platinum amine and amine complexes in aqueous solution [26–30]. In the preparation of *cis*-[Pt($^{15}NH_3$)$_2$(HFU)Cl], some minor amounts of *cis*-[Pt($^{15}NH_3$)$_2$(HFU)$_2$] and other impurities were also formed due to the inaccurate weighing and insufficient isolation, therefore, the recorded 2D [^{1}H, ^{15}N] HSQC NMR spectrum was rather complicated. The complexity mainly arose from the formation of *cis*-[Pt($^{15}NH_3$)$_2$(HFU)$_2$], which may be present in different tautomers as indicated above. In this case, the unambiguous assignment of all cross-peaks was difficult, but for some major cross-peaks, the most likely assignment was possible. Upon reacting the *cis*-[Pt($^{15}NH_3$)$_2$(HFU)Cl] solution with 5′-GMP, cross-peaks at 3.825/−69.936 ppm and 4.154/−69.888 ppm decreased in intensity with time, while cross-peaks at 4.220/−69.176 ppm and 4.230/−66.658 ppm increased in intensity. Therefore, the former peaks can be assigned to the $^{15}NH_3$*trans* to 5-FU and Cl^- of complex **1**, and the latter to $^{15}NH_3$ groups of the GMP adduct of **1** (Fig. 5, Table 4).

Once CDDP and 5-FU were combined together by a covalent bond, the interaction between them can be synergistic, i.e. the effect of the combined entity is larger than the algebraic sum of the effect of each agent separately [31], or be additive, i.e., the effect of the combination approximates the sum of the effect of each drug alone, or even be antagonistic, i.e., the combined effect of these two drugs is less than the sum of each agent. However, typical effects of these three interactions were not observed in our case, that is, the efficacy of the conjugated entity neither increased nor decreased remarkably. The antitumor activity of **2** against melanoma B16-BL6 cells is higher than that of **1**, especially at low

concentrations. This suggests that the antitumor mechanism of **2** is different from that of CDDP, and its antitumor activity arises from the 5-FU moiety rather than the CDDP moiety, because **2** is unable to bind DNA as shown in this work. According to the known structure–activity relationship of Cleare and Hoeschele, monofunctional platinum complexes are normally antitumor inactive due to the lack of ability to form bifunctional DNA adducts [32]. However, a series of *cis*-diammineplatinum (II) antitumor agents of the form *cis*-$[Pt(NH_3)_2(N\text{-donor})Cl]^+$, where the N-donor is a heterocyclic amine based on pyridine, pyrimidine, purine, or piperidine substituents, has been proved to be antitumor active [33]. The structural similarity of complex **1** to these complexes suggests that a similar antitumor activity and mechanism of action may exist. The potential mechanism of action may involve monofunctional covalent binding at N7 sites of guanine bases and intercalation of the planar nonleaving group (5-FU moiety) into the major grove of DNA to form 'pseudobifunctional' adducts. Such adducts would produce a disruptive and nonrepairable lesion on DNA. However, other mechanism of antitumor activity may operate in complex **1**. Further detailed experiments are required to clarify this problem.

5. Conclusion

The combination of 5-FU plus CDDP is the most beneficial chemotherapy regimen available for the treatment of patients with gastric cancer and head and neck cancer. Encouraged by this clinical fact, we synthesized 5-FU–CDDP conjugated complexes **1** and **2**, and investigated their reactivity towards 5′-GMP and their in vitro antitumor activities against melanoma B16-BL6 cells. Under our experimental conditions, 5-FU is able to coordinate with CDDP to form stable adducts. Complex **1** can further react with 5′-GMP, suggesting that it can still covalently bind to DNA. However, complex **2**, in which platinum was coordinated with four N-containing ligands (NH_3 or 5-FU), lost reactivity towards 5′-GMP under the same conditions.

The 5-FU–CDDP adducts did not show obvious superiority over 5-FU or CDDP in terms of in vitro activities. Because only one strain of tumor cells was tested in this work, it is not possible to make a more general evaluation on this type of combination, but the present work could potentially provide a novel alteration for the application of the two widely used clinical drugs.

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